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The Evolving Landscape of mRNA Therapeutics: From Molecular Design and Advanced Delivery Systems to Clinical Translation and Future Vistas

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Abstract: The messenger RNA (mRNA) therapeutic platform has transitioned from a theoretical concept to a clinically validated, transformative modality in contemporary medicine. The unprecedented success of the COVID-19 vaccines, which accelerated global recognition of this technology, has catalyzed an intensive and broad exploration across numerous therapeutic domains. This review provides an integrated examination of the molecular and engineering innovations that have underpinned the platform's maturation. Collective advances, including refinements in cap analog chemistry, the meticulous optimization of untranslated regions and the poly(A) tail, precise tuning of codon usage, and strategic nucleoside modification, have synergistically enhanced mRNA stability, translational efficiency, and immune tolerance. Concurrently, the parallel evolution of lipid nanoparticles (LNPs) design, specifically the development of ionizable and biodegradable lipids, has established a reproducible and safe framework for highly efficient *in vivo* delivery. Furthermore, the review considers next-generation constructs, such as circular RNA (circRNA) and self-amplifying RNA (saRNA), which hold the distinct promise of extending expression durability and substantially reducing required dosing regimens. Emerging applications now extend beyond vaccination to oncology, rare genetic and metabolic diseases, and immune modulation. Despite these advances, major challenges persist in achieving extrahepatic delivery, improving endosomal escape, and enabling thermostable, scalable manufacturing. Continued integration of molecular engineering, data-driven design, and nanotechnology is expected to transform mRNA into a modular, programmable therapeutic platform. Collectively, these developments position mRNA therapeutics at the forefront of precision and regenerative medicine, defining a new trajectory for next-generation biologics.

Keywords: messenger RNA; nucleoside modification; gene delivery; lipid nanoparticles; ionizable lipids; extrahepatic targeting.

1. Introduction

The transformation of mRNA from a theoretical therapeutic concept into a clinically validated platform represents one of the most significant paradigm shifts in modern medicine. Over three decades of persistent innovation have redefined the principles of drug development and vaccine design, inaugurating a new era of programmable therapeutics. The scientific foundation for this technology was established in the early 1990s, when Wolff *et al*. first

demonstrated that *in vitro*–transcribed (IVT) mRNA could drive protein synthesis *in vivo* [1], followed by the seminal study of Martinon *et al*., who demonstrated that mRNA encapsulated within liposomes could elicit cytotoxic T-lymphocyte responses against the influenza virus [2]. These pioneering discoveries established the conceptual framework for employing synthetic mRNA as a transient, non-integrating platform for therapeutic protein expression.

Despite such promise, the clinical translation of mRNA therapeutics was delayed for nearly two decades due to several intrinsic challenges. The molecule's inherent instability, rapid enzymatic degradation by ribonucleases, inefficient cellular uptake caused by its large anionic structure, and potent activation of innate immune sensors collectively hindered its development [3,4]. Unmodified mRNA was recognized as a pathogen-associated molecular pattern (PAMP), provoking inflammatory cascades that suppressed translation and induced toxicity. Overcoming these interconnected obstacles required integrated advances in molecular biology, chemistry, and nanotechnology to optimize RNA design, improve stability, and enable efficient intracellular delivery.

The pivotal breakthroughs that transformed mRNA into a viable therapeutic modality emerged from two convergent advances. The first centered on deliberate chemical optimization of the mRNA structure, particularly the substitution of native nucleosides with modified analogues, including pseudouridine (Ψ) and N1-methylpseudouridine (m1Ψ). These modifications attenuated innate immune recognition while simultaneously improving transcript stability and translational output [5–7]. The second advance was the development of sophisticated delivery systems, particularly lipid nanoparticles (LNPs), which protect the fragile RNA cargo, facilitate cellular internalization, and promote endosomal escape [8]. Together, these innovations provided the structural and functional foundation for modern mRNA therapeutics.

The clinical validation of this technology during the COVID-19 pandemic represented a defining moment for biomedical science. The rapid development, approval, and global deployment of the Pfizer–BioNTech (BNT162b2) and Moderna (mRNA-1273) vaccines demonstrated the unparalleled adaptability of the platform, combining speed of design with scalable, cell-free manufacturing [9,10]. Beyond infectious diseases, these achievements have accelerated the development of mRNA therapeutics for oncology, rare genetic disorders, regenerative medicine, and autoimmune diseases [11,12].

While several recent reviews have focused separately on the molecular design or delivery aspects of mRNA therapeutics, the present work offers a comprehensive, integrated perspective that links molecular engineering, advanced delivery technologies, and clinical translation. Beyond established approaches, this review highlights the ongoing evolution toward next-generation constructs such as circular RNA and self-amplifying RNA, together with emerging strategies for precise extrahepatic targeting, as the field advances toward tissue-specific and programmable RNA therapeutics. By uniting these perspectives, this review elucidates how successive innovations in molecular design, formulation technology, and clinical application collectively define the current landscape of mRNA therapeutics and shape their future trajectory.

2. Fundamental Challenges in mRNA Therapy

Despite remarkable progress, the widespread clinical application of mRNA therapeutics continues to face three fundamental and interconnected challenges: the intrinsic instability of the mRNA molecule, inefficient *in vivo* delivery, and unwanted immunogenic responses [13–

15]. These barriers, illustrated in Figure 1, represent critical bottlenecks that must be systematically addressed to realize the full therapeutic potential of mRNA technology across diverse disease applications.

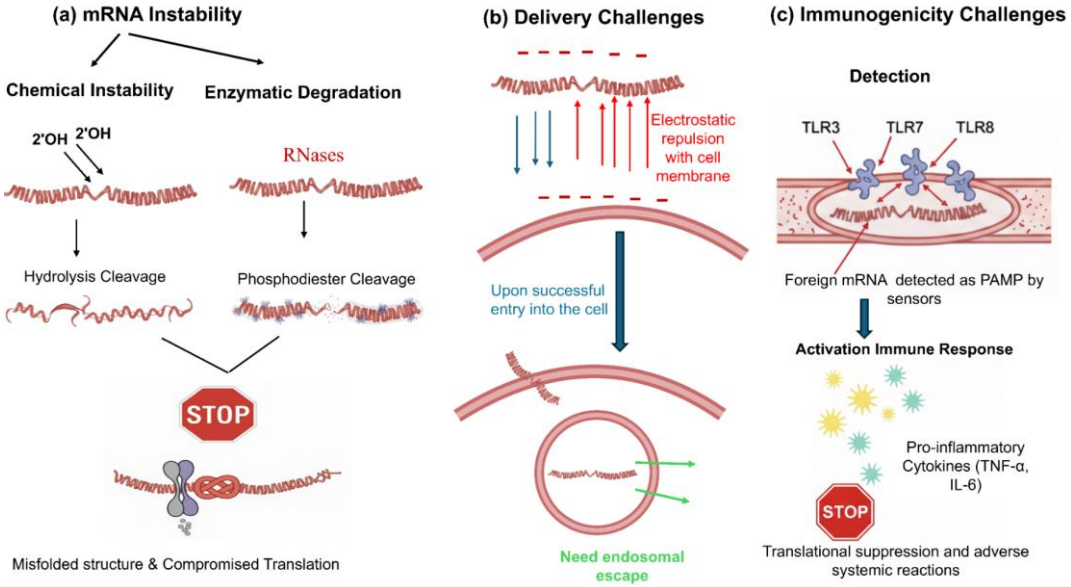

Figure 1. Barriers to mRNA therapeutics. **(a)** Instability: Messenger RNA (mRNA) undergoes chemical hydrolysis and enzymatic degradation by ribonucleases (RNases), reducing structural integrity and translation efficiency; **(b)** Delivery: Electrostatic repulsion limits cellular uptake, and endosomal entrapment restricts cytoplasmic release, emphasizing the need for efficient endosomal escape; **(c)** Immunogenicity: Toll-like receptors (TLRs) 3, 7, and 8 detect exogenous mRNA as a pathogen-associated molecular pattern (PAMP), inducing cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) that suppress translation and trigger inflammation.

2.1. mRNA instability.

The therapeutic success of mRNA depends on preserving its molecular integrity from administration to translation within target cells. Unmodified mRNA is highly susceptible to both chemical and enzymatic degradation: the 2′-hydroxyl group of ribose promotes hydrolytic cleavage, while extracellular and intracellular ribonucleases rapidly degrade the phosphodiester backbone [16]. In addition, disruption of secondary and tertiary structures impairs ribosome recruitment and translation efficiency, thereby limiting biological potency (Figure 1a) [17]. These vulnerabilities necessitate advanced delivery systems and stringent formulation controls, which directly influence mRNA pharmacokinetics and manufacturability.

2.2. Delivery barriers.

Efficient cytosolic delivery remains a principal obstacle to mRNA-based therapy. The molecule's large size and pronounced negative charge hinder its passive diffusion across the plasma membrane [18]. Nanoparticle-based carriers have therefore emerged as the dominant strategy for facilitating cellular uptake; however, the process remains constrained by endosomal entrapment and lysosomal degradation (Figure 1b) [19–21]. Only a small fraction of internalized mRNA escapes endosomes into the cytosol, necessitating higher therapeutic doses and increasing manufacturing costs, risks, and the risk of dose-dependent toxicity. Overcoming this delivery bottleneck remains a central focus of ongoing research, with novel

approaches aimed at modulating intracellular trafficking and nanoparticle–endosome interactions [22].

2.3. Immunogenicity.

The innate immune system is inherently primed to recognize foreign RNA as a viral signature. Exogenous mRNA containing unmodified nucleosides or double-stranded RNA (dsRNA) contaminants is detected as a PAMP by endosomal Toll-like receptors TLR3, TLR7, and TLR8, as illustrated in Figure 1c [23,24]. Activation of these pathways triggers the release of proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6), which can provoke systemic inflammation and suppress mRNA translation [25,26]. While controlled immunogenicity can be exploited to enhance vaccine efficacy, excessive or dysregulated activation compromises therapeutic safety and performance.

The interdependence among instability, delivery, and immunogenicity is a defining feature of mRNA therapeutic optimization. Protective carriers designed to shield mRNA from RNase degradation may inadvertently activate immune receptors, thereby influencing immunogenicity. Likewise, chemical modifications intended to minimize innate sensing can affect molecular stability and translational efficiency. The advancement of next-generation mRNA therapeutics, therefore, requires a systems-level strategy that balances these interrelated factors to achieve safe, stable, and clinically effective formulations.

3. Engineering the mRNA Molecule for Optimal Therapeutic Performance

The therapeutic performance of mRNA medicines is determined by the molecular design of the transcript. Advances in RNA chemistry and bioengineering have enabled precise optimization of stability, translation, and immunogenicity. Each structural element, including the cap, untranslated regions, coding sequence, and poly(A) tail, can be engineered to regulate expression kinetics, duration, and immune recognition, collectively defining the functional efficiency of therapeutic mRNA.

3.1. Strategies for enhancing mRNA stability, translational efficiency, and modulating immunogenicity.

The optimization of synthetic mRNA requires a comprehensive, multi-target approach that addresses distinct structural and chemical features of the molecule to achieve desired therapeutic outcomes.

3.1.1. The critical role of 5' cap structures and analogs.

The 5′ cap structure, composed of 7-methylguanosine linked to the first transcribed nucleotide through a triphosphate bridge (m^7GpppN), is indispensable for mRNA stability and translation. It protects transcripts from exonuclease degradation, facilitates recruitment of the eukaryotic initiation factor 4E (eIF4E), and ensures efficient ribosome loading during translation [27–29]. Progress in cap analog chemistry has substantially improved the performance of synthetic mRNA. Early capping methods often yielded reverse-oriented caps, resulting in reduced translational yield. The development of anti-reverse cap analogs (ARCAs) and the sulfur-substituted β-S-ARCA derivatives corrected this limitation by enhancing orientation fidelity and increasing resistance to decapping enzymes, thereby prolonging transcript half-life [30–34]. More recently, enzymatic and cotranscriptional capping

technologies, such as CleanCap®, have achieved near-complete capping efficiency and enabled the generation of Cap1 and Cap2 structures that closely resemble endogenous mRNA [35,36].

These advances have shifted the focus from purely chemical optimization to a more nuanced focus on biological functionality. Cap1 and Cap2 configurations not only enhance translational output but also reduce innate immune recognition through receptors such as retinoic acid-inducible gene I (RIG-I), thereby minimizing inflammatory activation [37]. The rational selection of capping strategies is thus a key determinant of therapeutic mRNA quality, ensuring a balance between manufacturability, translational potency, and immune tolerance.

3.1.2. Modulation of untranslated regions (UTRs) for regulatory control.

The UTRs flanking the coding sequence function as key regulatory domains governing mRNA stability, localization, and translational efficiency. The 5′ UTR is paramount in governing the mechanics of ribosome recruitment and the subsequent initiation dynamics [38–41]. Configurations that yield optimal translational output are invariably characterized by a concise, minimally structured sequence, coupled with the presence of a robust Kozak consensus motif, which collectively streamline the initiation phase. In contrast, the 3′ UTR operates as a sophisticated, multi-functional nexus for post-transcriptional regulation [42]. This region is densely populated with specific binding sites for RNA-binding proteins (RBPs) and microRNAs, whose coordinated actions precisely modulate the transcript's half-life and its ultimate translation into protein [43–45]. For example, the incorporation of AU-rich elements (AREs) or HuR-binding sites is a well-established mechanism for significantly extending mRNA's functional longevity and sustaining high levels of protein synthesis. A key strategy in therapeutic mRNA design involves the strategic deployment of UTRs derived from highly stable, naturally abundant transcripts, such as those encoding alpha-globin (α-globin) and beta-globin (β-globin), owing to their proven intrinsic stability and superior translational capacity [35,46,47]. This rational engineering of UTRs represents a powerful biotechnological lever, enabling precise control to tailor mRNA pharmacokinetics and pharmacodynamics. By meticulously customizing these non-coding segments, it becomes feasible to fine-tune both the magnitude and the duration of the resultant protein expression, thereby facilitating the development of therapeutic modalities that are either highly tissue-specific or strictly temporally regulated.

3.1.3. Poly(A) tail engineering for enhanced stability and translational efficiency.

The polyadenylate [poly(A)] tail, a homopolymeric sequence residing at the 3′ terminus of eukaryotic mRNA, stands as a non-negotiable structural element, the manipulation of which directly dictates transcript stability, translational efficiency, and the overall functional longevity of the molecule. Its critical function is mediated through the binding of poly(A)-binding proteins (PABPs), which, in turn, facilitate an essential interaction with the cap-associated eukaryotic initiation factor 4G (eIF4G) to establish the characteristic closed-loop mRNA conformation (Figure 2a). This specific, circularized architecture is the functional prerequisite for two key biological processes: it actively promotes the efficient, cyclical recycling of ribosomes for sustained protein synthesis, and, concurrently, it confers robust protection against the destructive kinetics of 3′-to-5′ exonucleolytic degradation [48–50].Poly(A) tail length represents a key tunable parameter in therapeutic mRNA design.

Empirical studies indicate that tails between 100 and 250 nucleotides generally yield optimal protein expression, although the precise range varies with cell type and expression kinetics [51]. Poly(A) sequences can be incorporated cotranscriptionally by encoding poly(T) tracts within the DNA template or posttranscriptionally using poly(A) polymerase, enabling controlled modulation of tail length and uniformity. During natural mRNA turnover, the poly(A) tail undergoes progressive shortening by cellular deadenylase complexes such as the carbon catabolite repressor 4-negative on TATA (CCR4–NOT) complex and the poly(A)-specific nuclease 2–poly(A)-specific nuclease 3 (PAN2–PAN3) complex. Tail shortening causes sequential loss of PABPs, disruption of the closed-loop structure, and eventual termination of translation (Figure 2b). To counteract this degradation pathway, engineered poly(A) tails containing non-adenosine residues such as cytidine or uridine have been developed (Figure 2c). These inserted nucleotides act as kinetic barriers that impede deadenylase progression, maintain tail length, and preserve cooperative PABPs–eIF4G interactions, thereby prolonging the translational lifetime of the mRNA [52–55].

These engineered architectures extend transcript half-life and enhance protein yield without appreciably increasing immunogenicity. When combined with optimized 5′ cap structures and translationally efficient untranslated regions, poly(A) tail engineering serves as a central determinant of mRNA pharmacological performance, enabling sustained and robust protein expression across diverse therapeutic applications.

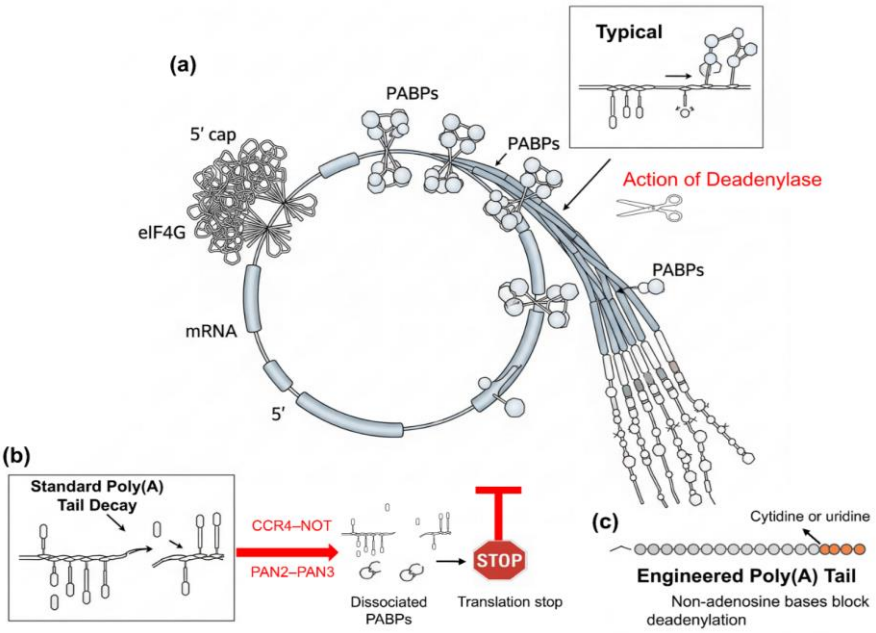

Figure 2. Poly(A) tail–mediated regulation of mRNA translation and stability. **(a)** Poly(A)-binding proteins (PABPs) associate with the 3′ poly(A) tail and the 5′ cap-bound eukaryotic initiation factor 4G (eIF4G), forming a closed loop that promotes ribosome recycling and protects the transcript; **(b)** Deadenylase complexes, including carbon catabolite repressor 4–negative on TATA (CCR4–NOT) and poly(A)-specific nuclease 2–poly(A)-specific nuclease 3 (PAN2–PAN3), progressivelyshorten the tail, leading to PABPs dissociation and translation stop; **(c)** Engineered poly(A) tails with non-adenosine nucleotides (cytidine or uridine; orange) resist deadenylase activity.

3.1.4. Impact of codon optimization and sequence composition.

The coding sequence influences mRNA stability and translational efficiency beyond its protein-encoding function. Codon optimization replaces rare codons with synonymous, host-preferred alternatives to improve translation elongation rates and reduce ribosomal pausing [56]. Balanced guanine–cytosine (GC) content enhances molecular stability through stronger

base-pairing interactions while avoiding excessive secondary structure that could impede ribosome progression [57,58]. Recent computational tools, including LinearDesign and mRNAdesigner, enable predictive sequence engineering by simultaneously optimizing codon usage, GC distribution, and folding thermodynamics [59]. This data-driven approach shifts mRNA sequence design from empirical trial-and-error to rational prediction, enabling scalable, reproducible manufacturing of high-performance therapeutic transcripts.

3.1.5. Chemical modifications of nucleosides and their impact.

The incorporation of chemically modified nucleosides has revolutionized RNA therapeutics by transforming highly immunogenic IVT RNA into a clinically tractable platform. As illustrated in Figure 3a, unmodified mRNA containing canonical uridine is readily detected by innate immune sensors, including TLR3, TLR7, and RIG-I. Their activation triggers type I interferon production, translational arrest, and the release of inflammatory cytokines [49,60]. Substituting uridine with modified analogues effectively attenuates these responses (Figure 3b). Among available modifications, N¹-methylpseudouridine ($m^1\Psi$) has emerged as the benchmark, enabling near-complete evasion of innate immune sensing while markedly enhancing translational efficiency and transcript stability. This principle underlies the clinical success of the Pfizer–BioNTech and Moderna mRNA vaccines [61,62].

Additional modifications, including pseudouridine (Ψ), 5-methoxyuridine (5moU), and 5-methylcytidine (5mC), provide intermediate degrees of immune modulation (Figure 3c). By varying the type and proportion of modified nucleosides, mRNA immunogenicity can be precisely tuned, ranging from strongly stimulatory profiles suited for vaccine adjuvanticity to minimally immunogenic configurations ideal for therapeutic protein expression [63–66]. Beyond immune modulation, nucleoside chemistry also improves physicochemical properties, such as resistance to ribonucleases, secondary-structure stability, and thermostability, thereby prolonging mRNA half-life and sustaining protein output under physiological conditions [67,68].

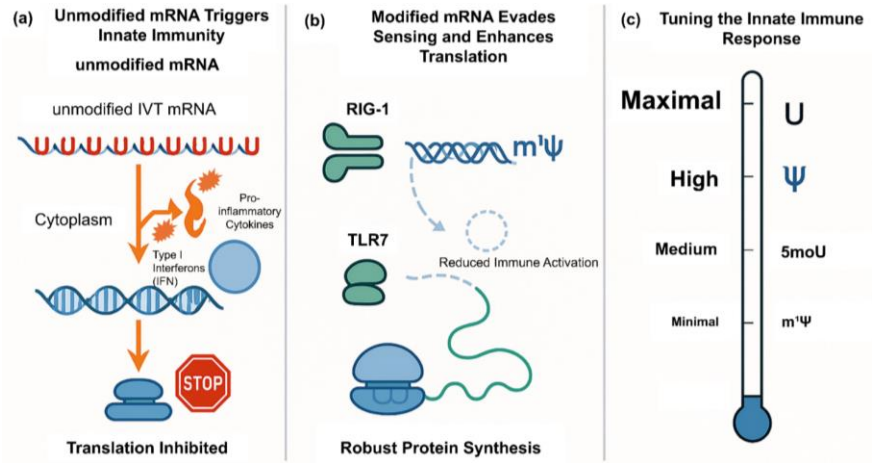

Figure 3. Nucleoside modifications regulate mRNA immunogenicity and translational output. **(a)** Unmodified in vitro–transcribed (IVT) mRNA containing uridine activates innate immune receptors, inducing type I interferon (IFN) production and pro-inflammatory cytokine release, which suppress translation; **(b)** mRNA incorporating N¹-methylpseudouridine ($m^1\Psi$) evades sensing by retinoic acid-inducible gene I (RIG-I) and Toll-like receptor 7 (TLR7), resulting in reduced immune activation and robust protein synthesis; **(c)** Different nucleoside modifications enable stepwise tuning of innate immune stimulation, ranging from maximal activation with uridine (U) to minimal activation with $m^1\Psi$, with pseudouridine (Ψ) and 5-methoxyuridine (5moU) providing intermediate responses.

Thus, strategic nucleoside modification provides a modular framework for balancing innate immune recognition, molecular stability, and translational potency, establishing a critical foundation for next-generation programmable RNA therapeutics.

Following the systematic optimization of individual structural domains, it is clear that the therapeutic performance of mRNA depends on the coordinated integration of all its modular components rather than any single molecular feature. The interplay among 5′ capping chemistry, UTRs architecture, poly(A) tail length and composition, codon usage, and nucleoside chemistry collectively determines the balance between translational efficiency, molecular stability, and innate immune recognition.

Table 1 summarizes these interdependent engineering strategies, outlining their mechanistic foundations, functional advantages, and inherent trade-offs that guide the rational design of therapeutic mRNA constructs. Collectively, these integrated molecular optimizations, further enhanced by computational and machine learning–based design tools, have transformed mRNA from a fragile experimental template into a programmable and clinically validated therapeutic platform [56,69].

Table 1. Comparative overview of mRNA engineering strategies highlighting their molecular advantages and translational trade-offs.

mRNA component	Strategy/modification	Primary mechanism	Advantages	Trade-offs / therapeutic implications	Ref.
5′ Cap Structure	Simple Cap Analog (m^7GpppN)	5′ protection and eIF4E recruitment	Basic stability; supports translation initiation	Moderate immunogenicity; limited translational output	[27-30]
	ARCA (3′-O-methyl-m^7G)	Correct orientation, improved eIF4E binding	Enhanced translation efficiency; better cap orientation	Slightly higher synthesis cost; moderate innate activation in some cell types	[31,32]
	β-S-ARCA (phosphorothioate)	Decapping enzyme resistance	Strongly improved mRNA half-life; higher protein yield	Synthetic complexity and cost	[33,34]
	CleanCap® (Cap1/Cap2)	Native-like mimicry and RIG-I evasion	Maximal translation and stability; clinically validated	Expensive reagents; specialized manufacturing	[35-37]
5′ UTR	Short, unstructured with Kozak consensus	Promotes efficient ribosome scanning	High translational efficiency, reproducible output	Over-shortening can reduce translational regulation capacity	[38-41]
	α/β-globin-derived motifs	Natural stabilizing elements	Strong stability and sustained expression	Less dynamic regulation under stress conditions	[35,46,47]
3′ UTR	ARE + HuR binding motifs	RBP-mediated stabilization	Improved half-life and translation in HuR-rich cells	Context-dependent performance; limited universality	[43-45]
	α/β-globin 3′ UTR	Endogenous stability motifs	Maximized stability and expression; widely used scaffold	May limit rapid turnover when transient expression is desired	[35,46,47]
Poly(A) Tail	Standard poly(A) (100–250 nt)	PABP binding and mRNA circularization	Balanced translation and stability; compatible with most cell types	Moderate half-life; susceptible to deadenylation	[48-51]
	Length optimization	Tail length tuning for translational efficiency	Maximized ribosome recycling and expression control	Excessively long tails may trigger innate sensing	[51]
	Non-A insertions (C/U)	Deadenylase resistance	Extended transcript lifetime; sustained translation	May alter PABP interaction; requires optimization	[52-55]
Coding Sequence	Codon optimization	Host-preferred codon usage	Maximized translation speed and yield	Over-optimization can impair co-translational folding	[56]
	GC balancing and structure control	Secondary structure tuning	Enhanced stability and balanced expression	Excess GC can slow elongation and reduce expression	[57-59]

mRNA component	Strategy/modification	Primary mechanism	Advantages	Trade-offs / therapeutic implications	Ref.
Nucleoside Modification	Unmodified uridine (U)	Recognized by TLRs and RIG-I	Natural template; simple synthesis	Highly immunogenic; short half-life; not suitable for therapy	[49,60]
	Pseudouridine (Ψ)	Partial immune evasion	Increased stability and translation; moderate immune modulation	Intermediate immunogenicity; application-specific	[16,65,66]
	5-Methoxyuridine (5moU)	Weakened innate sensor recognition	Reduced inflammation; moderate translation	Limited data on long-term safety; niche applications	[61]
	5-Methylcytidine (5mC)	Duplex stabilization and immune attenuation	Increased mRNA stability and translational output	Modest benefit when used alone; often combined with Ψ or $m^1\Psi$	[35]
	N^1-methylpseudouridine ($m^1\Psi$)	Complete immune evasion, enhanced translation	Highest protein output and long half-life; clinically validated	Costly synthesis but gold-standard modification for vaccines and therapeutics	[63,64]

3.2. Next-generation RNA constructs.

The continued maturation of RNA therapeutics has catalyzed the development of next-generation RNA platforms designed to overcome the inherent limitations of conventional linear mRNA. Among these, circRNA and saRNA have emerged as the most advanced architectures. Each offers a distinct strategy to prolong protein expression, reduce required dose, and modulate innate immune activation, thereby expanding the functional and therapeutic versatility of RNA-based medicines (Figure 4).

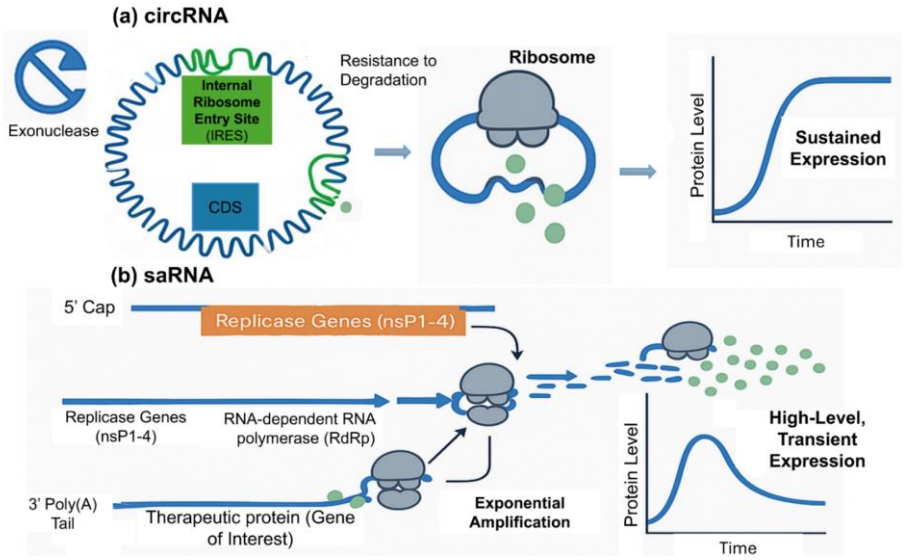

Figure 4. Next-Generation mRNA Architectures for Controlled Expression. **(a)** Circular RNA (circRNA): A covalently closed loop enhances stability by resisting exonuclease degradation, enabling sustained protein expression suitable for therapeutic protein replacement; **(b)** Self-Amplifying RNA (saRNA): Encodes a viral replicase to amplify the mRNA template, producing a high-level, transient expression from a low dose.

3.2.1. circRNA.

Circular RNA (circRNA) molecules form covalently closed single-stranded loops that lack 5′ and 3′ termini, rendering them highly resistant to exonuclease-mediated degradation and enabling sustained protein translation through engineered internal ribosome entry sites (IRESs) (Figure 4a) [70]. These molecules exhibit markedly prolonged intracellular half-lives (19–48 hours) compared with linear mRNA (4–9 hours) [71–73].

Despite these advantages, the clinical translation of circRNA remains constrained by manufacturing challenges. Production typically involves two sequential steps: *in vitro*

transcription of a linear precursor followed by intramolecular ligation. The ligation reaction is intrinsically inefficient, in part due to competing intermolecular events that generate linear concatemers and double-stranded RNA impurities. Consequently, advanced purification workflows, including RNase R digestion combined with high-performance liquid chromatography (HPLC), are required to achieve pharmaceutical-grade purity [74]. Even minor process variations, such as trace magnesium ion contamination, can reduce purification efficiency. These impurities compromise product yield and introduce safety concerns, as unligated RNA and double-stranded species can activate innate immune pathways. The consistent, large-scale production of highly pure circRNA that meets regulatory expectations for biologic therapeutics, therefore, remains a key barrier to clinical translation.

Although no circRNA-based therapeutics have yet received regulatory approval, multiple clinical candidates and a growing number of preclinical programs are in development worldwide. The RXRG001 Phase I/II study (NCT06714253), which evaluates an aquaporin-1 circRNA therapy for radiation-induced xerostomia, demonstrates the feasibility of prolonged *in situ* protein replacement. Additional preclinical efforts in oncology and neurodegeneration leverage the inherent stability and sustained expression profile of circRNA. Collectively, these properties position circRNA as a promising platform for applications requiring durable, low-immunogenic protein production, including enzyme replacement therapy and chronic protein supplementation [75–77].

3.2.2. saRNA.

Self-amplifying RNA (saRNA), derived from positive-sense RNA viruses such as alphaviruses, incorporates a replicase cassette encoding non-structural proteins 1 to 4 (nsP1–nsP4), which mediate intracellular amplification of the target transcript (Figure 4b) [78]. Following delivery, translation of these non-structural proteins forms an active replicase complex that generates multiple subgenomic RNA copies encoding the therapeutic antigen, enabling high-level protein expression from microgram-scale doses. Preclinical studies have demonstrated dose-sparing efficiencies of up to an order of magnitude compared with conventional mRNA vaccines, translating into reduced manufacturing demand and potentially lower reactogenicity [79].

Clinically, saRNA has shown translational promise through candidates such as ARCT-154, which elicited durable neutralizing antibody responses against COVID-19, and GEMCOVAC-OM, which achieved protection against circulating Omicron-lineage variants [80,81]. GEMCOVAC-OM is particularly notable for its lyophilized formulation, which enables storage at 2–8°C and supports distribution in settings without ultracold infrastructure [82,83]. These attributes underscore the platform's suitability for scalable, globally accessible vaccine deployment.

Despite these advantages, several limitations must be addressed. saRNA constructs are comparatively large (10–15 kb versus 1–5 kb for conventional mRNA), which complicates encapsulation within LNPs and reduces formulation efficiency at scale [84]. Viral replication within the cytosol produces long double-stranded RNA intermediates that activate innate immune sensors such as protein kinase R (PKR) and RIG-I. Although this intrinsic adjuvanticity can enhance vaccine potency, excessive signaling may trigger systemic reactogenicity and suppress translation. Additionally, expression of the viral replicase proteins may induce adaptive immune responses, potentially limiting tolerability during repeated dosing. Unlike chemically modified mRNA, saRNA cannot incorporate nucleoside

modifications, such as N¹-methylpseudouridine, limiting its ability to mitigate innate immune activation in applications requiring chronic or repeated delivery.

To overcome these challenges, the trans-amplifying RNA (taRNA) system has emerged as a promising alternative. This platform divides the replicase and antigen-encoding sequences into two separate RNA molecules, reducing overall transcript length, simplifying nanoparticle formulation, and enabling independent modulation of amplification and antigen expression [85]. These properties position taRNA as a compelling candidate for repeat vaccination and for gene therapy applications requiring controlled, sustained, and well-tolerated protein production.

4. Advanced Delivery Systems for Therapeutic mRNA

The clinical performance of mRNA therapeutics relies heavily on the development of delivery systems that protect the transcript and enable efficient cytoplasmic entry. Owing to its large size and strong negative charge, mRNA is rapidly degraded by extracellular nucleases and cannot cross cellular membranes without a protective carrier, necessitating its use. Among the diverse delivery platforms explored, including polymeric nanoparticles and exosomes, LNPs have emerged as the most advanced and clinically validated. Their success in the first U.S. Food and Drug Administration (FDA)–approved small interfering RNA (siRNA) therapeutic, patisiran, and in the COVID-19 mRNA vaccines firmly establishes LNPs as the foundational technology for modern nucleic acid delivery [86].

4.1. Fundamentals of lipid nanoparticle design.

LNPs are typically spherical structures measuring 80–120 nm and are composed of four key components, namely ionizable lipids, helper phospholipids, cholesterol, and polyethylene glycol (PEG)–lipids (Figure 5). Ionizable lipids, which constitute approximately 46–50 mol% of the formulation, are the primary drivers of mRNA encapsulation and endosomal escape. Their tertiary amine headgroups have an apparent pKa of 6.0-7.0 and remain neutral at a physiological pH of about 7.4, thereby minimizing cytotoxicity and reducing nonspecific serum interactions.

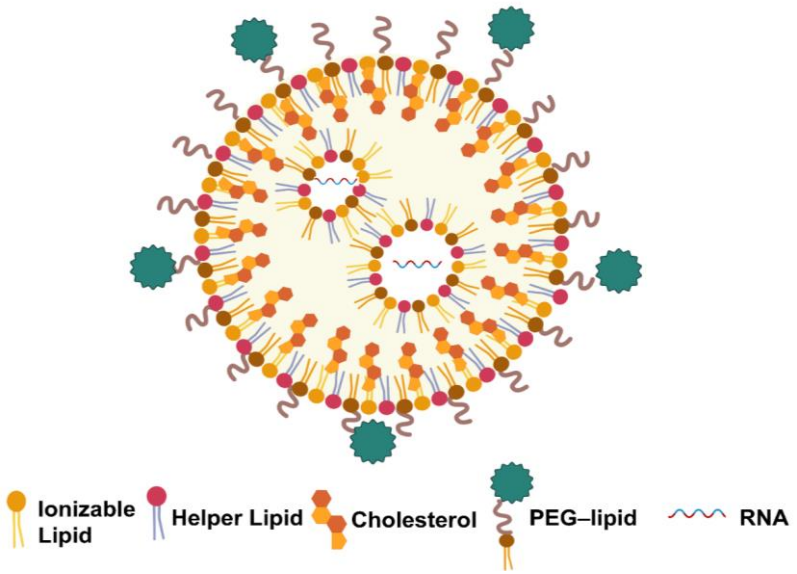

Figure 5. Schematic representation of a typical lipid nanoparticle (LNP) for RNA delivery, illustrating the four core lipid components: an ionizable lipid, a helper lipid, cholesterol, and a polyethylene glycol (PEG) lipid, which together form a stable nanostructure that encapsulates and protects the mRNA cargo.

In the acidic endosomal environment (pH 5.0-6.5), these groups become protonated, enabling electrostatic interactions with the negatively charged mRNA backbone and facilitating destabilization of the endosomal membrane for cytoplasmic release [86–88]. Helper phospholipids, generally present at 10–12 mol%, maintain bilayer organization and facilitate membrane fusion. Common examples include 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), which promotes endosomal escape through its ability to adopt non-bilayer phases, and 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), which enhances structural rigidity and improves colloidal stability [89]. Cholesterol, typically comprising 38–43 mol%, modulates membrane fluidity and mechanical strength, thereby preventing leakage and increasing formulation stability [90]. PEG–lipids (1–2 mol%) generate a hydrophilic corona that limits aggregation, reduces opsonization, and prolongs systemic circulation by minimizing clearance via the mononuclear phagocyte system [91–93]. The specific ratio of these components determines particle size, encapsulation efficiency, and *in vivo* performance.

4.2. Formulation and manufacturing.

The evolution of LNPs formulation from empirical mixing toward a controlled engineering discipline has been central to the clinical translation of mRNA therapeutics. Microfluidic manufacturing enables precise and scalable production by rapidly combining a lipid solution in ethanol with an aqueous buffer containing mRNA under acidic conditions. This environment promotes spontaneous self-assembly of nanoparticles with uniform size distribution and high encapsulation efficiency [94–97].

Recent advances, including antifouling microchannel coatings and the integration of process analytical technology (PAT), now enable real-time monitoring of critical particle attributes, such as size, concentration, and encapsulation efficiency. Integrating these tools within a Quality by Design (QbD) framework supports consistent batch performance and alignment with regulatory requirements [98–101]. Together, these innovations have transformed LNPs manufacturing into a robust, reproducible, and globally scalable platform that enables widespread deployment of mRNA-based therapeutics.

4.3. Biological fate and mechanistic delivery pathway.

After administration, LNPs rapidly adsorb circulating plasma proteins to form a dynamic protein corona that defines their biological identity and biodistribution. In particular, adsorption of apolipoprotein E (ApoE) facilitates receptor-mediated uptake by hepatocytes, explaining the intrinsic liver tropism observed for most formulations [102,103]. While advantageous for hepatic indications, this tropism limits extrahepatic delivery and has motivated the development of alternative lipid chemistries to redirect tissue targeting.

Cellular internalization of LNPs is mediated by several endocytic pathways, including clathrin-mediated endocytosis, caveolae-mediated endocytosis, and macropinocytosis [104,105]. As illustrated in Figure 6, internalized nanoparticles are trafficked to early endosomes, where lumenal acidification to approximately pH 6.2 initiates partial protonation of ionizable lipid headgroups. Continued maturation to late endosomes further lowers the pH to approximately 5.0, enhancing electrostatic interactions between the positively charged, ionizable lipids and anionic phospholipids, such as phosphatidylserine. These interactions destabilize the endosomal membrane, enabling a fraction of the encapsulated messenger RNA

to escape into the cytoplasm [106–108]. Nanoparticles failing to escape during this maturation window are ultimately trafficked to lysosomes, where enzymatic degradation occurs.

Despite high levels of cellular uptake, endosomal escape remains the major efficiency bottleneck, with fewer than 2% of internalized nanoparticles achieving productive cytosolic delivery. Ongoing research focuses on rational lipid design, including branched hydrophobic tails and fusogenic helper phospholipids, to enhance escape while maintaining biocompatibility [109–111].

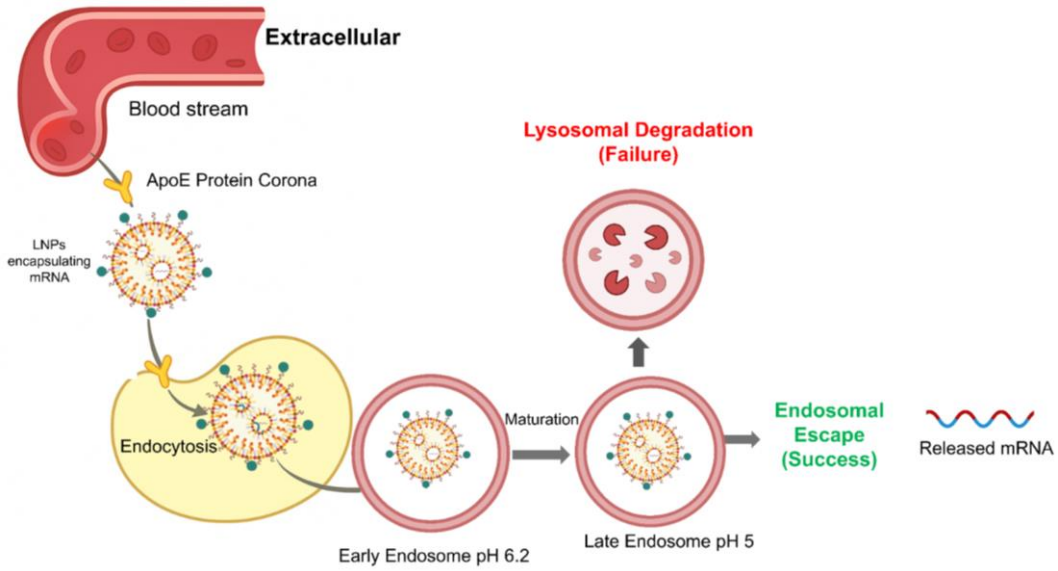

Figure 6. Biological pathway of lipid nanoparticles (LNPs) encapsulating messenger RNA (mRNA). Following systemic administration, adsorption of apolipoprotein E (ApoE) forms a protein corona that facilitates cellular uptake through endocytosis—internalized LNPs traffic from early to late endosomes during vesicular maturation. Particles that successfully escape the endosomal compartment release their mRNA payload into the cytosol, whereas those failing to escape are routed to lysosomes for enzymatic degradation.

4.4. Advances in ionizable lipid design for mRNA delivery.

Ionizable lipids form the molecular foundation of LNPs and are the primary determinants of mRNA delivery efficiency, stability, and safety. Their physicochemical properties govern all stages of intracellular trafficking, including nucleic acid encapsulation, endosomal escape, biodistribution, and metabolic clearance. Early benchmark lipids established essential parameters for potency and manufacturability. Current engineering efforts now focus on biodegradability, reduced immunogenicity, and organ-selective targeting, reflecting the field's transition from empirical formulation to rational, mechanism-guided design.

4.4.1. Clinical benchmarks established by Vanguard Lipids.

The first clinically validated ionizable lipid, DLin-MC3-DMA (MC3), marked a major milestone in nucleic acid therapeutics. Developed by Alnylam Pharmaceuticals and Acuitas Therapeutics, MC3 enabled the approval of patisiran, the first FDA-approved siRNA therapy [112]. Its tertiary amine headgroup and dilinoleyl tails confer an apparent pKa of approximately 6.4, supporting hepatic uptake and efficient endosomal protonation. Despite its strong performance in liver-targeted delivery, MC3 undergoes slow biodegradation due to its chemical stability, leading to prolonged tissue residence and mild, dose-dependent

hepatotoxicity at higher exposures [113,114]. These limitations informed the design of next-generation lipids with faster clearance.

The ionizable lipids SM-102 (used in Moderna Spikevax) and ALC-0315 (used in Pfizer–BioNTech Comirnaty) represent the second generation of clinically validated lipids that define current mRNA vaccine platforms [115–117]. Both incorporate biodegradable ester linkages that undergo enzymatic hydrolysis, addressing the slow metabolic clearance associated with MC3 and improving safety during repeated dosing [118]. SM-102 has an apparent pKa of about 6.7, which enhances endosomal protonation and promotes robust cytosolic release, resulting in strong antigen expression after intramuscular (IM) administration. ALC-0315, with an apparent pKa near 6.1, provides a more balanced profile of potency and tolerability, enabling high translation efficiency with relatively low local reactogenicity. Although these lipids have demonstrated excellent performance, they remain optimized primarily for transient IM delivery rather than systemic administration, and their long-term biodegradation kinetics are not yet fully characterized [119]. Together, they highlight the design trade-offs required to balance potency, clearance, stability, manufacturability, and safety.

4.4.2. Next-generation lipids for biodegradability and advanced function.

Modern ionizable lipid engineering increasingly relies on modular, rational design guided by structure–activity relationships. The incorporation of ester or other hydrolysable linkers shortens tissue residence time and improves safety during repeated dosing [120]. The candidate 5D8, containing an ester backbone identified through Michael-addition combinatorial screening, demonstrates high gene-editing activity with minimal hepatotoxicity [121].

Linker chemistry has emerged as a tunable determinant of organ tropism. Urea and reverse-amide linkers, exemplified by Lipid 35 and Lipid 36, promote preferential accumulation in the lung [122]. Tetrahydropyrimidine (THP) headgroups increase potency while reducing cytokine induction [123]. Hydroxyl-rich (HTO) headgroups strengthen hydrogen bonding with mRNA, enabling lower lipid-to-mRNA ratios and improving formulation efficiency [124].

Optimization of the hydrophobic tail further expands performance capabilities. Branched-tail (BEND) architectures improve membrane destabilization and endosomal escape [125]. Sterol-integrated lipids, such as CS22021, incorporate cholesterol-like groups into the ionizable framework, concentrating expression at the injection site and preferentially stimulating CD8+ T-cell responses, which is advantageous for cancer vaccine applications [126]. High-throughput combinatorial libraries have also yielded dendron-like and siloxane-containing lipids with programmable biodistribution, enabling selective mRNA expression in tissues such as the spleen and lung [127–129]. In parallel, iso-A11B5C1, identified through Passerini three-component reaction (P-3CR) combinatorial synthesis, demonstrates muscle-specific delivery with markedly reduced off-target expression in the liver and spleen [130,131]. Collectively, these advances illustrate the shift from liver-restricted formulations toward chemically programmable LNP platforms capable of organ-selective delivery and controlled biodegradation.

Table 2. Comparative summary of representative ionizable lipids for LNPs-mRNA delivery.

Category	Representative lipid	Distinctive structural feature	Apparent pKa	Primary target/route	Key functional advantage	Translational context	Ref.
Clinically validated	DLin-MC3-DMA (MC3)	Tertiary amine headgroup with dilinoleyl tails	~6.4	Liver intravenous (IV)	High potency for hepatic delivery; robust endosomal activation	Patisiran (Onpattro); FDA-approved siRNA therapy	[113,114]
	SM-102	Ester-containing hydrophobic tail (heptadecan-9-yl ester)	~6.7	IM	Strong endosomal protonation; potent antigen expression	Spikevax (Moderna); approved mRNA vaccine	[115–117]
	ALC-0315	Hydroxybutyl amine core with esterified tails	~6.1	IM	Balanced potency and tolerability; controlled biodegradation	Comirnaty (Pfizer–BioNTech); approved mRNA vaccine	[119]
Emerging	5D8	Ester backbone for rapid hydrolysis	—	Liver	High gene-editing efficiency with improved clearance	Preclinical; enhanced biodegradability	[121]
	Lipid 35 / 36	Urea or reverse-amide linker	7.6 / 7.2	Lung	Linker-defined pulmonary tropism; tumor suppression potential	Preclinical targeted delivery studies	[122]
	THP1	Tetrahydropyrimidine headgroup	~5.4	Liver gene editing (IV); mRNA vaccines (IM route)	Scalable catalyst-free multicomponent synthesis; high mRNA transfection efficiency; sustained expression; low toxicity	Liver-targeted gene editing and mRNA vaccine delivery in preclinical models	[123]
	CS22021	Sterol-integrated core	—	mRNA vaccine (IM injection)	Localized mRNA expression; robust cytotoxic T lymphocyte ($CD8^+$ T-cell) response; improved safety	Varicella-zoster virus (VZV) mRNA vaccine model; potential platform for $CD8^+$ T-cell–oriented cancer vaccines in preclinical studies	[126]
	18-2-9b2	Dendron-like multi-arm architecture	—	Spleen	Protein-corona-driven macrophage tropism	Demonstrated targeted mRNA and genome editing in splenic macrophages (Ai14 mice) in preclinical studies	[127]
	iso-A11B5C1	Ester-based lipid from P-3CR combinatorial chemistry	—	Muscle cells (IM)	Exceptional muscle-selective mRNA delivery; minimal off-target transfection (liver/spleen/antigen-presenting cells)	Demonstrated muscle-specific mRNA transfection, muscle gene editing, and potent antitumor effect in melanoma models (preclinical)	[130,131]

4.4.3. Comparative and translational summary.

Table 2 summarizes representative ionizable lipids, emphasizing their defining chemical features, apparent pKa values, delivery routes, and translational significance. The progression from stable amine-based scaffolds to biodegradable ester-linked and sterol-integrated frameworks illustrates how iterative chemical refinement enhances potency, safety, and tissue selectivity.

4.4.4. Interpretive perspective.

The developments summarized in Table 2 illustrate a clear shift from early liver-targeted ionizable lipids to next-generation materials with programmable biodegradation and organ-selective properties. MC3 established the first therapeutic benchmark but lacked rapid metabolic clearance. SM-102 and ALC-0315 demonstrated that efficient biodegradability can be achieved at a clinical scale with strong safety performance. Emerging lipids extend this framework by revealing how changes in linker chemistry, headgroup architecture, or tail structure influence potency, immune activation, and tissue distribution.

Important challenges remain. The relationships between specific chemical motifs and *in vivo* biodistribution are not yet fully understood, and the long-term fate of many lipid degradation products remains to be fully characterized. Integrating high-throughput synthesis with machine learning prediction and quantitative proteomic profiling will be essential to transition lipid chemistry from empirical optimization to predictive molecular engineering. Ultimately, the field is moving toward customizable LNPs that deliver mRNA precisely to intended tissues with minimal toxicity and high translational reliability.

4.5. Expanding beyond the liver: strategies for extrahepatic targeting.

4.5.1. The challenge of hepatotropism.

Following systemic administration, LNPs naturally accumulate in the liver due to the organ’s fenestrated sinusoidal endothelium and the adsorption of ApoE, which mediates receptor-dependent uptake by hepatocytes [87]. This intrinsic hepatotropism benefits hepatic gene therapies and systemic protein production platforms but limits the application of LNPs for extrahepatic targets. Overcoming this bias remains one of the most formidable barriers in mRNA therapeutics. While several chemical and physical strategies have been proposed, achieving reproducible, predictable biodistribution across species and disease models continues to be a central translational challenge.

4.5.2. Endogenous and passive targeting strategies.

Endogenous or passive targeting seeks to redirect LNPs' biodistribution through internal design modifications rather than surface ligands. Among these, Selective Organ Targeting (SORT) represents a major conceptual breakthrough. Incorporating a fifth lipid component into standard formulations enables controlled alterations in surface charge and protein corona composition, yielding distinct organ tropisms. For example, permanently cationic SORT lipids promote lung delivery, whereas anionic species, such as phosphatidic acid, drive spleen accumulation [132]. This modular design principle allows systematic tuning of biodistribution without changing core composition or formulation methods.

Chemical tailoring of the ionizable lipid itself further influences tropism. Urea or reverse-amide linkers induce strong lung selectivity [122], whereas modification of hydrophobic tail length or branching shifts delivery toward spleen and lymphoid tissues. Variations in PEG-lipid density, chain length, and nanoparticle size (<80 nm) can also refine circulation time and tissue penetration [133–136]. Despite their elegance, passive targeting strategies remain highly empirical, with outcomes strongly dependent on animal model, dose, and formulation method. Developing predictive computational or proteomic models of the LNPs–protein corona interface will be crucial for rational, cross-species design of next-generation organ-selective carriers.

4.5.3. Active targeting strategies.

Active targeting strategies incorporate molecular recognition elements that guide LNPs toward defined cell populations. Conjugation of antibodies or antibody fragments, including fragment antigen binding (Fab) regions and single-chain variable fragments (scFvs), to PEG lipids enables receptor-mediated uptake by immune cells and tumor cells [137]. However, conventional chemical conjugation often produces heterogeneous ligand orientations and variable surface densities, which can reduce targeting efficiency and complicate reproducibility and large-scale manufacturing. To address these challenges, modular and conjugation-free systems have been developed. Bispecific antibodies can bridge peptide-tagged LNPs to specific cell-surface receptors, such as programmed death ligand 1 (PD-L1) or cluster of differentiation 4 (CD4), enabling flexible, highly selective targeting [138]. Antibody–fragment crystallizable (Fc) fusion proteins provide uniform, oriented surface coatings that enhance targeting precision. For instance, formulations targeting human epidermal growth factor receptor 2 (HER2) have enabled selective delivery of tumor suppressor protein p53 (p53) mRNA, resulting in complete tumor regression with minimal hepatic exposure in preclinical models [139].

Smaller ligands, including peptides, aptamers, and nanobodies, provide superior tissue penetration and reduced immunogenicity, expanding the repertoire of non-antibody targeting approaches. Emerging evidence supports a multimodal strategy combining endogenous tropism, such as spleen-directed SORT LNPs, with receptor-specific ligands to achieve cell-level precision. This approach has enabled in situ generation of chimeric antigen receptor T cells (CAR-T cells) within lymphoid tissues, demonstrating a significant advance in in vivo cellular engineering [140].

A key remaining challenge is achieving stable ligand presentation with consistent orientation and manufacturing uniformity at scale. Translational progress will require standardized and modular conjugation chemistries that balance targeting precision with regulatory feasibility.

4.5.4. Local and advanced delivery modalities.

Beyond molecular engineering, the administration route can profoundly influence organ specificity. Inhaled or nebulized LNPs formulation allows localized pulmonary delivery for diseases such as cystic fibrosis or lung cancer, maximizing tissue exposure while minimizing systemic toxicity [141]. For neurological disorders, intracerebroventricular (ICV) or intrathecal administration bypasses the blood–brain barrier (BBB), enabling direct access to neurons and glia. A transformative non-invasive strategy involves focused ultrasound (FUS)

coupled with microbubbles to transiently and reversibly open BBB junctions [142–144]. This approach enhances nanoparticle extravasation and local mRNA expression in targeted brain regions, as demonstrated in preclinical glioblastoma and neurodegeneration models [145–147]. Despite encouraging results, the durability and spatial reproducibility of FUS-mediated BBB opening require further clinical standardization. Integration with real-time imaging and safety biomarkers will be essential for scalable CNS translation.

4.6. Overcoming practical hurdles toward broad clinical translation.

4.6.1. Improving stability beyond cold-chain constraints.

mRNA's intrinsic instability and the susceptibility of LNPs to aggregation have imposed stringent storage requirements. Early COVID-19 vaccines required ultra-cold storage (−20°C to −80°C), limiting global distribution [17]. Lyophilization (freeze-drying) with cryoprotectants such as sucrose or trehalose has since enabled long-term stability at 4°C for over a year, with full restoration of biophysical and biological properties upon rehydration [148]. Optimizing cholesterol and phospholipid ratios further improves post-lyophilization integrity and thermostability [149]. However, large-scale lyophilization remains cost-intensive and time-consuming. Future research must focus on liquid-stable or self-stabilizing formulations, potentially through adaptive lipid chemistries or glass-forming excipients, to eliminate the need for cold-chain logistics.

4.6.2. Mitigating PEG-related immunogenicity.

Repeated administration increases the risk of immune reactions against PEG lipids, driven by pre-existing or treatment-induced anti-PEG antibodies (APAs) [150–153]. Although these responses are typically transient in vaccine settings, the induction of APAs poses significant challenges for chronic mRNA therapies that require repeated dosing [154]. Mitigation strategies include substituting methoxy-terminated PEG with hydroxyl-terminated analogues, which exhibit reduced antibody binding [155], and employing high-density brush polymer architectures that limit complement activation [156,157].

Next-generation alternatives, such as polysarcosine (pSar) and zwitterionic polymers, exhibit stealth behavior with negligible immunogenicity while maintaining or improving delivery efficiency [158–161]. Despite these advances, the long-term biocompatibility and biodegradation profiles of PEG substitutes remain insufficiently defined. Establishing standardized preclinical frameworks that assess complement activation, opsonization kinetics, and cumulative immune responses will be essential for regulatory acceptance of PEG-free LNPs.

4.7. The expanding clinical landscape.

Propelled by the success of COVID-19 vaccines and continuous innovation in lipid nanoparticle design, the clinical pipeline for mRNA therapeutics is rapidly expanding across diverse disease areas, demonstrating the platform's remarkable versatility.

4.7.1. Infectious disease applications.

The clinical maturation of mRNA technology is most prominently demonstrated in the realm of infectious disease prophylaxis, a field where the platform's inherent adaptability and

capacity for expedited development have proven transformative [162]. Building on the foundational success of the SARS-CoV-2 vaccines, current research is increasingly focused on developing multivalent and combination formulations that confer simultaneous immunity against multiple respiratory pathogens via a single inoculation. This is exemplified by the Phase III clinical evaluation of dual-target candidates, such as Moderna's mRNA-1083 and the Pfizer–BioNTech COMIRNATY formulation, which co-encode antigens for both SARS-CoV-2 and seasonal influenza viruses. These combined vaccines are strategically designed to streamline annual immunization protocols, thereby enhancing patient adherence and mitigating the overall burden on healthcare infrastructure [162]. Preliminary clinical findings confirm the induction of robust neutralizing antibody titers against both influenza A and SARS-CoV-2; however, the comparatively diminished response against influenza B strains underscores the need for continued refinement of antigen design to ensure comprehensive and balanced epitope coverage [163].

Furthermore, the scope of next-generation mRNA vaccines extends significantly beyond conventional respiratory targets, with rapid progress being made against emerging and re-emerging infectious diseases. The unpredictable emergence and rapid dissemination of these pathogens frequently overwhelm traditional vaccine platforms, which are often constrained by limitations in manufacturing speed and scalability. The inherent modularity and cell-free synthesis of the mRNA- LNPs system position it as an exceptionally well-suited technology for mounting an expeditious response to newly identified or rapidly mutating infectious agents [164]. Preclinical data powerfully illustrate this broad-spectrum potential across diverse etiological systems. For instance, mRNA-LNPs vaccines encoding the fusion glycoprotein F of respiratory syncytial virus (RSV-F) have been shown to elicit potent $CD4^+$ and $CD8^+$ T-cell activation, conferring durable protection that demonstrably surpasses that achieved by protein-based comparators [165]. Similarly, constructs expressing the outer surface protein A (OspA) from Borrelia burgdorferi have generated robust immunity against bacterial infection in animal models [166], thereby confirming that the utility of mRNA-LNPs technology is not restricted solely to viral targets. Programmes focused on non-viral pathogens such as malaria and tuberculosis further highlight the value of thermostable and lyophilised formulations for deployment in resource-limited regions. Collectively, these advances establish mRNA vaccines delivered by LNPs as a rapidly maturing platform for infectious disease prevention. Their combination of programmable antigen design, scalable manufacturing, and efficient immune activation now rivals that of established vaccine modalities, positioning mRNA LNPs as a cornerstone of future global vaccination strategies.

4.7.2. Oncological applications.

Oncology has become one of the most transformative frontiers for mRNA therapeutics, representing a shift from prophylactic vaccination to personalised immunotherapy. In this context, mRNA LNPs vaccines act as therapeutic agents that activate the immune system to recognise and eliminate malignant cells [167]. LNPs deliver mRNA encoding tumour-associated antigens or patient-specific neoantigens. After uptake by antigen-presenting cells, the translated proteins are presented via major histocompatibility complex (MHC) class I and II pathways, triggering $CD8^+$ cytotoxic and $CD4^+$ helper T-cell responses [168]. The same molecular optimisations that improve stability and translation—nucleoside modification, refined cap structures and ionisable lipids—are critical for achieving durable antitumour immunity.

Translation into oncology remains limited by the immunosuppressive tumour microenvironment, heterogeneous antigen expression, and T-cell exhaustion. To overcome these challenges, LNPs are being bioengineered to serve as both delivery systems and immune modulators. Modern formulations incorporate adjuvant-encoding mRNAs or innate immune receptor agonists, such as TLR and RIG-I ligands, to enhance dendritic cell activation and T-cell priming. Advances in lipid chemistry, including biodegradable ester linkages and sterol-modified lipids, have further improved intratumoral mRNA translation while maintaining systemic tolerability [169]. Combination regimens have become integral to clinical development. The administration of mRNA LNPs vaccines together with immune checkpoint inhibitors, including PD-1 or cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) antibodies, enhances T-cell activation and reduces adaptive resistance. Other strategies combine vaccination with adoptive T-cell transfer or radiotherapy to promote antigen release and immune infiltration within the tumour microenvironment.

Although no mRNA-based cancer vaccine has yet received regulatory approval, numerous Phase I and II clinical trials have demonstrated encouraging safety and immunogenicity, even in tumours previously regarded as poorly responsive to immunotherapy. Current programmes include personalised neoantigen vaccines, fixed-antigen constructs, and mRNA-engineered CAR T-cell therapies [164]. Table 3 summarises representative mRNA LNPs oncology candidates in clinical development, describing their targets, administration routes, and translational significance.

Table 3. Representative mRNA–LNPs cancer therapeutics in clinical development.

Candidate / Sponsor	Target or Mechanism	Cancer Type / Route	Phase (2025)	Key Translational Feature	Trial ID	Ref.
Autogene cevumeran (BNT122) – BioNTech + Genentech	Personalized neoantigens	Resected high-risk pancreatic or colorectal cancer (IV/IM)	Phase II	Durable polyclonal T-cell responses; delayed recurrence in pancreatic cancer	NCT04486378	[167, 168]
mRNA-4157 (V940) – Moderna	Personalized neoantigen vaccine	Resected high-risk melanoma (IM vaccine with IV pembrolizumab)	Phase II	Improved recurrence-free survival compared with pembrolizumab monotherapy	NCT03897881	[170]
FixVac BNT111 – BioNTech	Fixed set of tumour-associated antigens (TAAs) – melanoma	Advanced melanoma (IV RNA-lipoplex)	Phase I	Induces $CD4^+$ and $CD8^+$ responses; demonstrates partial responses in early trials	NCT02410733	[171]
BNT113 – BioNTech	Human papillomavirus type 16 (HPV16) E6 and E7 oncoproteins	HPV16-positive head and neck cancer (IM)	Phase II	Viral-oncogene targeting; synergy with checkpoint blockade	NCT04534205	[172]
BNT116 – BioNTech	Fixed antigen set for non-small-cell lung cancer (NSCLC)	Advanced/metastatic NSCLC (IM)	Phase I	Lung cancer-targeted mRNA vaccine across global sites	NCT05142189	[171]
CV9202 – CureVac	Six shared tumour antigens (NSCLC)	Advanced or metastatic NSCLC (IM) administered alone or in combination with the checkpoint	Phase I/II	Multi-antigen approach; strong antigen-specific T-cell responses and a feasible combination with	NCT03164772	[27]

Candidate / Sponsor	Target or Mechanism	Cancer Type / Route	Phase (2025)	Key Translational Feature	Trial ID	Ref.
		inhibitors durvalumab and tremelimumab		dual immune checkpoint blockade		
BNT211 (CARVac) – BioNTech	Claudin-6 (CLDN6) CAR-T mRNA with vaccine support	Claudin-6-positive advanced solid tumours treated with *ex vivo* mRNA-transfected T cells administered IV and *in vivo* mRNA vaccine boosting	Phase I/II	First mRNA-engineered CAR-T platform targeting solid tumours; transient CAR expression enhances safety; vaccine component prolongs CAR-T activity	NCT04503278	[173]
mRNA-4359 – Moderna	PD-L1 + indoleamine 2,3-dioxygenase 1 (IDO1) immunomodulation	Advanced solid tumours (IM)	Phase I/II	Tumour-microenvironment re-education; checkpoint synergy	NCT05533697	[174]
iNeST/BNT114 – BioNTech	Multiple antigens + p53 mRNA	Triple-negative breast cancer (IM)	Phase I	Broad-antigen strategy in breast cancer combined with immunotherapy	NCT02316457	[164]

4.7.3. Rare genetic disease applications.

mRNA–LNPs therapeutics have shown considerable promise for monogenic disorders characterised by absent or dysfunctional protein expression. In cystic fibrosis (CF), inhaled LNPs formulations such as ARCT-032 (Arcturus Therapeutics) and VX-522 (Vertex Pharmaceuticals and Moderna) deliver mRNA encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein directly to the airway epithelium, restoring chloride transport in preclinical models and demonstrating encouraging activity in early clinical evaluation [175,176]. ARCT-032 is currently being assessed in a Phase II study (NCT06747858). A complementary strategy is represented by mRNA-3705 (Moderna), which encodes methylmalonyl-CoA mutase (MUT) for the treatment of methylmalonic acidemia (MMA). Intravenous administration enables hepatic enzyme expression and has produced favourable biochemical correction in an ongoing Phase I/II clinical trial (NCT04899310) [177–179]. Another advanced program, mRNA-3927, delivers mRNA for both subunits of propionyl-CoA carboxylase to treat propionic acidemia and is being evaluated in a Phase I/II study (NCT04159103). In contrast, mRNA-3351 for Crigler–Najjar syndrome type I, which encodes the bilirubin-metabolising enzyme uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1), remains in preclinical development with no registered clinical trial to date.

Although these programmes demonstrate meaningful therapeutic potential, applying mRNA platforms to rare genetic diseases presents distinctive challenges. Many monogenic conditions require sustained protein restoration in tissues that are difficult to target with current LNPs, including skeletal muscle, the central nervous system, and specialised metabolic organs. Furthermore, because these disorders frequently affect paediatric populations, long-term safety, developmental pharmacokinetics, and the feasibility of chronic administration must be carefully optimised. Collectively, these advances mark a shift from symptomatic management toward direct molecular restitution through transient mRNA replacement. Continued progress in organ-targeted delivery, LNPs chemistry, and chronic-use safety will be essential to fully realise the therapeutic potential of mRNA medicines for rare genetic disorders.

4.7.4. Autoimmune disease applications.

An emerging therapeutic strategy involves using LNPs for *ex vivo* cell engineering in the treatment of autoimmune disorders. In this approach, highly efficient LNPs are used to deliver mRNA encoding the master regulatory transcription factor Forkhead box P3 (FoxP3) into primary human T cells, thereby converting them into regulatory T cells (Tregs) with potent immunosuppressive activity [49,180]. These engineered Tregs have the potential to modulate pathogenic immune responses that drive autoimmune and inflammatory diseases. This LNPs-based method offers higher transfection efficiency and lower cytotoxicity than conventional viral vector systems and may be adaptable to a wide spectrum of conditions, including allergic diseases and transplant rejection.

5. Conclusions

The evolution of mRNA therapeutics illustrates how advances in molecular design and delivery engineering have collectively reshaped modern medicine. Improvements in cap analog chemistry, untranslated region optimization, codon composition, and nucleoside modification have transformed mRNA from a fragile transcript into a stable and translationally efficient therapeutic modality. Concurrent refinement of LNPs technology, particularly in ionizable lipid chemistry and controlled microfluidic manufacturing, has enabled reproducible and clinically validated delivery. Together, these innovations have established mRNA as a versatile platform with applications ranging from infectious disease prevention to oncology, genetic disorders, and immune modulation.

Despite this progress, several challenges continue to limit the full therapeutic potential of mRNA. The inherent hepatic tropism of LNPs restricts extrahepatic delivery, and inefficient endosomal escape remains a major intracellular barrier. Issues related to molecular stability, repeat dosing, and innate immune activation further complicate long-term treatment strategies. Moreover, reliance on cold-chain logistics and the complexity of large-scale manufacturing hinder equitable global access. Addressing these limitations will require coordinated efforts that integrate molecular engineering, computational modeling, and advanced materials science to achieve predictable biodistribution, efficient intracellular trafficking, and sustained safety profiles.

Future development will depend on next-generation RNA constructs and delivery systems designed to enhance precision, durability, and tissue specificity. Circular RNA, self-amplifying RNA, and trans-amplifying RNA offer new opportunities for prolonged expression and reduced dosing. Advances in lipid chemistry, particularly biodegradable and organ-selective formulations, together with data-driven insights into nanoparticle–protein interactions, are enabling rational design of targeted delivery systems. Integration of artificial intelligence, high-throughput screening, and proteomic mapping is accelerating predictive engineering and reducing reliance on empirical optimization. The translational scope of mRNA therapeutics now extends beyond vaccination to regenerative medicine, oncology, and genetic correction. Their capacity to transiently express therapeutic or immunomodulatory proteins without genomic integration provides a controlled means to modulate biological pathways. Recent achievements in *in situ* generation of CAR-T cells and induction of regulatory T cells demonstrate the emerging potential of RNA-driven cellular reprogramming. As RNA chemistry, nanotechnology, and immunoengineering continue to intersect, mRNA therapeutics

are transitioning from emergency-use applications to a central component of precision medicine.

Overall, the advancement of mRNA therapeutics reflects a broader shift in biomedical science toward design-driven molecular engineering. The integration of sophisticated RNA chemistry with innovative delivery technologies has transformed instability into precision and innate immunogenicity into programmable immune modulation. The coming decade will determine whether these achievements culminate in a modular, widely accessible RNA platform capable of addressing both global infectious threats and individualized genetic diseases. Realizing this vision will depend on sustained interdisciplinary collaboration among chemistry, computation, and clinical translation to ensure that the promise of RNA therapeutics matures into a durable, transformative reality for human health.

Author Contributions

Conceptualization, E.S. and S.E.M.; methodology, E.S. and S.E.M.; data curation, E.S.; formal analysis, E.S.; writing—original draft preparation, E.S.; writing—review and editing, E.S., A.A.F., and S.E.M.; supervision, S.E.M. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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